## C.2 Technical Abstract

### Protocol K-0015 titled "A Phase I/II Study of Vaccination with Irradiated Autologous Lung Tumor Cells Mixed with a GM-CSF Secreting Bystander Cell Line (Lung Bystander GVAX®) in Advanced Stage Non-small Cell Lung Cancer (NSCLC)".

This study is designed to investigate the use of autologous, lethally irradiated non-small-cell lung (NSCLC) cells admixed with a bystander cell engineered by plasmid gene transfer to secrete human granulocyte-macrophage colony-stimulating factor (GM-CSF) as a therapeutic vaccine. Phase I/II studies of vaccination with autologous. irradiated. cultured tumor cells, engineered by retroviral or adenoviral-mediated gene transfer to secrete human GM-CSF. have been performed in melanoma, renal cell carcinoma, lung, and prostate cancer. Phase I/II studies of allogeneic GM-CSF–modified tumor cell vaccines have also been performed in prostate and pancreatic cancer. These studies have revealed the consistent development of potent antitumor immunity without the induction of significant toxicity. We are now investigating a new vaccine platform in NSCLC. We plan to evaluate the use of an allogeneic bystander GM-CSF–producing cell (K562 Bystander GVAX® mixed with autologous tumor cells as the vaccine formulation. The final vaccine is referred to as Lung Bystander GVAX.

Lung cancer is the leading cause of cancer death for men and women in the United States. Non-small-cell lung carcinoma (NSCLC) accounts for *75%* to 80% of these cases. Although the incidence of this disease may be declining in American men. it has been steadily increasing in American women, following the steady rise in tobacco use. The worldwide increase in tobacco consumption, moreover, is likely to be associated with a global increase in lung cancer deaths during the 21st century.

#### OBJECTIVES

The primary objectives are to evaluate the safety of Lung Bystander GVAX, and to evaluate the feasibility of vaccine preparation using a mixture of autologous lung tumor cells and K562 Bystander GVAX. The secondary objective is to evaluate the immunologic and clinical responses to vaccination with Lung Bystander GVAX.

#### PATIENT POPULATION

Approximately 40 adults with histologically confirmed NSCLC stage IIIA, IIIB, or IV.

#### STUDY DESIGN

Phase I/II, open-label, multicenter, multidose.

#### TREATMENT PLAN AND SCHEDULE

Eligible patients will undergo a tumor harvest procedure to obtain tumor cells for vaccine manufacturing. The cells will be processed, irradiated, and frozen. The anticipated success rate of autologous lung tumor cell processing is approximately 80%. If tumor cell processing is successful, vaccination will start approximately one month after tumor procurement. Patients will be actively participating in this study for approximately 14 months, including approximately 4 to 6 weeks between tumor procurement and vaccination and a 6 to 24-week vaccination period. At the time of vaccination, specified dose of autologous tumor cells and K562 Bystander GVAX cells will be thawed, mixed, and administered as intradermal injections. Patients will be followed for one year following the first vaccination and thereafter will be followed for survival for a total of five years.

#### DOSE

Five dose levels ranging from $5 \times 10^6$ to $8 \times 10^7$ autologous tumor cells admixed with K562 Bystander GVAX cells at a constant tumor to bystander ratio of 2:1 have been selected for the present study.

Dose-escalation will be in 2-fold increments, and a total of 3 to 12 vaccinations will be administered intradermally at 2-week intervals. Patients will be assigned to each dose level dependin[g] on tumor cell yields rather than order of entry into the trial.

**ENDPOINTS**

The primary endpoints of the trial are feasibility of vaccine processing and safety of vaccine administration. Safety monitoring will include measurement of standard laboratory toxicity and assessment of adverse events. In addition, patients enrolled at the two hi[g]hest dose levels ≥ 40 ng/106 cells/24 hr GM-CSF) will undergo daily monitoring of serum GM-CSF levels for 4 days following the first and third vaccination to assess the in-vivo survival and GM-CSF secretion from vaccine cells. Secondary endpoints include assessment of immunologic and clinical response to vaccination. Immunologic monitoring will include visual and histologic assessment of delayed-type hypersensitivity reaction to injections of autologous, irradiated tumor cells and measurement of humoral responses to vaccination. Clinical responses will be assessed through standard radiologic evaluations.

**PRODUCT**

K562 Bystander G V A X is a mixture of autologous tumor cells and GM-CSF–secreting K562 "bystander" cells. The tumor cells will be obtained from the patient's NSCLC tumor cells, enriched by density gradient centrifugation. irradiated at 10,000 rads to prevent cell growth *in vivo*, frozen in autologous plasma and DMSO without any *ex-vivo* expansion, and stored in vapor-phase liquid nitrogen prior to use. K562 Bystander G V A X consists of K562 erythroleukemia cells en[g]ineered by plasmid transfection to secrete human GM-CSF. In addition to GM-CSF, the plasmid utilized (pCep) contains the gene for ampicillin-resistance, hygromycin-resistance, and the E B N A - 1 origin of replication sequence from Epstein-Barr Virus. K562 cells do not express HLA class I/II antigens and are, therefore, unlikely to trigger a direct T-cell–mediated anti-K562 immune response. K562 Bystander G V A X cells are grown in suspension, in X-Vivo 20 serum-free medium containing 1.2 mg/mL Hygromycin B, irradiated at 10,000 rads to arrest cell growth, formulated in a cryoprotectant, and frozen in liquid nitrogen. The product is supplied in single-use 1.2-mL vials, each containing $3 \times 10^7$ cells. At the time of vaccination, the tumor cells and K562 Bystander G V A X® cells will be thawed and mixed at a ratio of 2 tumor cells to 1 K562 Bystander G V A X cell. This will constitute the final vaccine formulation (Lung Bystander G V A X).

This study is closed to enrollment and all vaccine treatments have been completed.